THE COMPLEMENT FIXATION REACTION IN THE DIAGNOSIS OF CONTAGIOUS ABORTION OF CATTLE.*

W. P. LARSON.†

(From the Wisconsin Experiment Station and Agricultural College, University of Wisconsin.)

The disease commonly known as contagious abortion of cattle has been known to veterinarians and stock breeders for nearly a century. It was during the early part of the nineteenth century that the disease was first noticed in England and France; but it was not until about the year 1850 that it was recognized as a contagious disease.

In the year 1878 Lehnert made a study of the disease, at that time very prevalent in the European countries devoted to stock breeding. He was the first to call attention to the fact that a healthy heifer would abort after having received a vaginal injection of uterine exudate from an animal suffering from contagious abortion.

Braüer likewise succeeded in terminating pregnancy prematurely in cows, by inserting into the vagina placental fragments taken from animals which had recently aborted.

By means of these experiments the transmissibility of the disease was established. Its true pathology was not understood at this period, however, as it was generally supposed that it was a constitutional disease rather than a local one.

The work of Nocard, although unfruitful in its effort to isolate the microorganism responsible for the widespread disease, threw valuable light on its pathological nature. He concluded from his studies that contagious abortion was not a disease which affected the general health of the animal materially, but that it was confined exclusively to the uterine mucosa, the fetus and fetal membranes. Pathologists of today are able to add but little to the teachings of this venerable scientist.

It was in the year 1896 that Bang and Stribolt took up the problem which had now apparently rested for a period of nearly 10 years. These authors were the first to isolate the microorganism which is now considered as the specific contagium of abortion in cattle.

Having procured an animal which presented typical clinical symptoms of threatening abortion, they made preparations for the elaborate study which resulted in the discovery of the long-sought-for microbe.

The technic followed by Bang and Stribolt was the following:

The animal which they had chosen for their study was killed. The gravid uterus was then carefully enucleated and transferred to the laboratory under aseptic precautions.

*Received for publication October 30, 1911.

† Demonstrator of Pathology and Bacteriology, University of Minnesota.

Tubes containing gelatin serum agar were inoculated from the amniotic fluid according to the ordinary method of anaerobic culture. The inoculated tubes were then placed at a temperature of 37°. After a period of from two to four days they noticed small colonies almost imperceptible to the naked eye developing just beneath the surface of the medium; this zone of growth varied from 1–2 cm. in depth. These colonies they described as being round, very small, some not larger than a needle point, others as large as a pin head. Examined under the microscope the edges of the colonies appeared serrated.

The microorganism thus isolated was found to be a cocco-bacillus measuring from 1–2 μ in length. It is non-spore-bearing, stains readily with the ordinary anilin dyes, and is gram negative.

Upon studying the biological properties of this cocco-bacillus further, Bang and Stribolt were impressed by its peculiar behavior toward oxygen. In view of the fact that the colonies developed just beneath the surface of the culture medium, the bacillus was designated as a semi-anaerobe, requiring for its development an environment in which the oxygen pressure was less than that of the ordinary atmosphere. This characteristic was found to be peculiar to the first generation only; the succeeding generations, although able to grow in ordinary atmosphere, were found to thrive best in an atmosphere of nearly pure oxygen.

Successful in their attempts to precipitate abortion in cows, sheep, and goats, with either intravenous or intravaginal injections of a pure culture of the cocco-bacillus, Bang and Stribolt concluded that this was the specific etiological agent of contagious abortion.

Following the technic recommended by their Danish contemporaries, McFadyean and Stockman of Great Britain, and Nowak of Austria soon succeeded in isolating an organism identical with that described by Bang and Stribolt.

McFadyean and Stockman also developed a method for the isolation of the cocco-bacillus of Bang which, in their hands, gave better results than the one above described. This consisted in growing a culture of *B. subtilis* in a closed chamber together with plates inoculated with material containing *B. abortus*. The culture of *B. subtilis*, in absorbing a part of the oxygen contained in the chamber, created an atmosphere favorable to the development of *B. abortus*.

Strange as it appears, American bacteriologists hitherto have not been as successful in isolating this organism as their European colleagues, though recently McNeal and Kerr have isolated a microorganism which they believe to be identical with the bacillus of Bang.

In view of this fact the opinion has been gaining credence in America that the specific agent responsible for the epizootic abortion in this country is not identical with that of Europe. The question of identity, therefore, was the first to arrive in the conduct of the investigation here recorded. It seemed probable that it could be solved by the complement fixation reaction, using a Copenhagen culture of *B. abortus* as antigen. The kindness of

Dr. Holth of the Royal Veterinary Laboratory of Copenhagen supplied the culture and this work was made possible. I wish to express my appreciation of the assistance given me by Dr. Holth.

The antigen consisted of a 10-day serum broth culture which was prepared as follows: 200 c.c. of ordinary broth, to which was added 50 c.c. of naturally sterile horse serum, were placed in a sterile Erlenmeyer flask of 750 c.c. capacity. The flask was closed by means of a rubber stopper perforated by two glass tubes, A and B, well plugged with cotton. Tube A extended deep into the culture medium to within 1 cm. of the bottom of the flask. Tube B merely perforated the stopper.

The flask was then placed at a temperature of 37° C. for 24 hours. If at the end of this time no bacterial growth developed it was inoculated with *B. abortus* and immediately oxygenated. The oxygen was gently led through a cotton filter into tube A for a period of about five minutes. The tubes A and B were then carefully sealed with paraffin, and the flask placed at a temperature of 37° C. for a period of 10 days. The antigen was then ready for use. The antigen thus prepared, if carbolized, will keep for months.

Hemolysin was prepared by injecting a rabbit, intraperitoneally, with 7 c.c. of a 1 per cent suspension of washed horse corpuscles, at intervals of five days.

On the 10th day following the fifth injection, the hemolytic titer of the serum was determined. It was found that 0.05 c.c. of a 1 per cent solution of serum was sufficient to hemolyze 0.5 c.c. of a 1 per cent suspension of washed horse corpuscles within 45 minutes.

The rabbit was then killed, and the blood, which was collected under aseptic precautions, defibrinated and centrifuged.

The serum, after being apportioned in hermetically sealed pipettes of about 0.5 c.c. each, was inactivated at 56° for half an hour and placed in the ice-chest to be kept for further use.

Guinea-pig serum served as complement. The titer of this complement and of the antigen was determined as indicated in the following tables:

Tubes	Guinea-Pig Serum (Dilution 1–4)	Hemolysin 1 per cent	Red Corpuscles—Horse 1 per cent	NaCl Solution 9 gm. per Liter	Degree of Hemolysis
1	....	....	0.5	1.5	0
2	0.03	0.15	"	"	0
3	0.04	"	"	"	Partial
4	0.06	"	"	"	Complete
5	0.08	"	"	"	"
6	0.10	"	"	"	"

The titer of the complement in this case lies between 0.04 and 0.06 c.c. In the presence of antigen-serum of a normal animal (cow), 0.04–0.06 c.c. complement would not be sufficient to effect complete hemolysis. In practice it is therefore necessary to use more than this amount of complement.

The amount of complement necessary varies according to the nature and amount of antigen used. In the present investigation it has been found practical to use twice the titer of the complement, for which has been adopted the term "titer dose." In the instance here cited the titer dose would be 0.1 c.c. (2×0.05).

The following table illustrates the technic used in titration of antigen:

Tubes	Antigen	Complement (Dilution 1–4)	Hemolysin 1 per cent	Red Corpuscles—Horse 1 per cent	Inactivated Serum of Infected Animal (Cow)	NaCl Solution	Degree of Hemolysis
1	0.0	0.1	0.15	0.5	0.02	1.5	Complete
2	0.4	"	"	"	"	"	0
3	0.3	"	"	"	"	"	0
4	0.2	"	"	"	"	"	0
5	0.1	"	"	"	"	"	0
6	0.05	"	"	"	"	"	0
7	0.02	"	"	"	"	"	Partial
8	0.01	"	"	"	"	"	Complete

Thus it is seen that at least 0.05 c.c. antigen is required to inhibit hemolysis in the presence of the specific amboceptor (serum of infected cow).

In examining the sera of cattle to determine whether or not they are infected with contagious abortion, the procedure adopted was the following:

Tubes	Antigen	Complement (Dilution 1-4)	Hemolysin 1 per cent	Red Corpuscles 1 per cent	Suspect Serum Inactivated	NaCl Solution	Degree of Hemolysis
1.....	0	0	0	0.5	0	1.5	0
2.....	0	0.1	0.15	"	0.02	"	Complete
3.....	0.15	"	"	"	0	"	"
4.....	"	"	"	"	0.02	"	0
5.....	"	0	"	"	"	"	0
6.....	"	0.1	"	"	"	"	0
7.....	"	"	"	"	0.01	"	0
8.....	"	"	"	"	0.005	"	Partial

The above table represents a positive reaction.

In practicing the complement deviation reaction it is necessary to add to the tubes containing the specified amount of NaCl solution, the antigen, the suspect serum, and the complement; this being done the tubes are placed in the thermostat at 37° for one hour in order to allow the complement time to become fixed. At the end of the hour the hemolysin and red corpuscles are added, and the tubes again placed in the thermostat and left for two hours. At the end of this time the reaction is noted.

Up to the present time there has been occasion to examine for the purposes of this study the sera of 76 animals. In the following table is submitted a brief history of each animal and the result of the examination:

History	Number	Reaction
Never aborted.....	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 47	−
Infected herd:		
Never aborted.....	51, 52, 53, 67, 69, 70	−
" "	50 (two-year-old)	+
" "	49 (three-year-old; calved once)	+
" calved.....	41 (two-year-old)	−
" "	43, 45, 46 (two-year-olds)	+
Aborted one year ago.....	54, 56	+
" " " "	55	−
Aged, aborted years ago.....	44	−
Aborted three times in succession.	57	+
Carried calf full period	15 (calf died 15 minutes after birth)	+
One-year-old.....	68	−
Aborted 1910.....	71, 72, 73, 74, 75, 76, 63, 65	+
" "	64 (now 5 mos. pregnant)	+
Aborted 1911.....	4 (January 30), 42 (May 29)	−
" "	17 (March 10), 18 (January 2), 19 (February 19), 38 (March 10), 40 (August 1), 39 (February 19)	+
Aborted 1910 and August 2, 1911...	66	+
Aborted last two years.....	62, 60 (now 5 mos. pregnant)	+
Aborted.....	58 (last year), 59 (3 years ago)	+
" one year ago	48	+
" three years ago.....	61 (since carried 2 calves to term)	+
Calf 4½ mos. old, born prematurely.	21 (dam reacts)	−

The results shown in the table are to be summarized as follows:

Of the 76 animals examined, 32 gave a positive reaction and 44 a negative. Of the 32 which gave a positive reaction, 25 have aborted. Six animals which reacted have never aborted. These were all without exception in infected herds. One animal from an infected herd which reacted carried fetus full term, but calf died 15 minutes after birth.

Of the 44 animals which reacted negatively, two have aborted. These two abortions may have been due to other causes. One of these two animals was in an infected herd, the other not. Of the 44 animals which gave a negative reaction, 10 were in infected herds.

While this work was being carried on attempts were made to isolate the etiological agent of contagious abortion in this country. Thus far seven fetuses from different sections of the state have been examined.

The procedure was as follows:

Tubes of liquefied 1 per cent agar were placed in a water bath at 55° for 15 minutes. After this time there was added to the agar one-fifth its volume of naturally sterile horse serum at the same temperature. The tubes of serum-agar thus formed were then cooled to 42°, and inoculations were made from the gastro-intestinal tract of the fetus. The inoculated tubes were immediately placed in cold water, to insure a rapid solidification of the culture medium.

Tubes of slant serum agar were likewise inoculated from the gastro-intestinal tract of the fetus, and placed in a closed chamber together with cultures of *B. subtilis*. This is a modification of the method recommended by McFadyean and Stockman.

Both of these procedures were successful in cultivating a cocco-bacillus which corresponds in detail, culturally and morphologically, to the cocco-bacillus first described by Bang and Stribolt. The organism was isolated from five of the seven fetuses examined.

It was further found in this investigation that this cocco-bacillus would grow very well on the surface of slant serum agar provided care was exercised to seal the tubes hermetically immediately after inoculation. The culture medium, which had previously been

deprived of oxygen by being heated, undoubtedly absorbs a sufficient amount of the oxygen contained in the tube to create an atmosphere favorable to the development of the bacillus abortus.

It has been said that the organism in question will usually be found in pure culture in the gastro-intestinal tract of the fetus. In this study contamination was found to be the rule.

In order further to establish the identity of the cocco-bacillus which had thus been successfully isolated, resort was had once more to the complement fixation reaction, this time, however, the cocco-bacillus isolated as described above being used as antigen.

For this work 15 animals were chosen, the sera of which had previously been examined, using the Copenhagen culture as antigen. Nine of these animals had given a positive reaction, and six a negative.

The reactions in these two series coincided in every instance. These experiments then, it may be believed, have proved beyond question that the European disease and the American disease are identical.

CONCLUSIONS.

1. Contagious abortion of cattle in this country is caused by a microorganism identical with that causing the disease on the European Continent.

2. The complement fixation reaction is a reliable and accurate method of diagnosis.

3. All animals do not contract the disease, even if in an infected herd and living under the same conditions as those which become infected.

4. An animal may react positively, indicating that she has at some period been infected, and yet may not abort. This brings up the question of immunity, which will be the subject of a future study.

I wish to take this opportunity of expressing my thanks to Dr. F. B. Hadley for the assistance he has rendered me.

BIBLIOGRAPHY.

BANG, B. *Maanedskrift f. Dyrlaeger*, 8 and 10; *Ztschr. f. Tiermed.*, 1897, 1, p. 241.
BRÄUER. *Säch. Veterinärber*, 1880, pp. 72, 76.
HOLTH, H. *Maanedskrift f. Dyrlaeger*, 1911, 22, p. 1.
HOLTH, H. *Berl. tierärztl. Wchnschr.*, 1909, 25, p. 686.
GRINSTAD. *Maanedskrift f. Dyrlaeger*, 1910, 21.
ISAACHSEN. *Kalvekastning, dens Behandling og Forebygyelse*, Kristiana, 1905.
KOLLE AND WASSERMANN. *Handbuch* 3, p. 827.
LEHNERT. *Säch. Veterinärber.*, 1878, p. 95.
MACNEAL, W. J., AND KERR, JOSEPHINE E. *Jour. Infect. Dis.*, 1910, 7, p. 469.
MCFAYDEAN AND STOCKMAN. *Report of the Committee to Inquire into Epizootic Abortion*, London, 1909.
NOCARD. *Rec. de Méd. Vét.*, 1886, p. 689.
NOWAK. *Ann. de l'Inst. Pasteur*, 1908, 22, p. 541.
WALL, SR. *Maanedskrift f. Dyrlaeger*, 1910, 21.
WILCOX, E. V. *Am. Vet. M. Ass. Exper. Sta. Rec.*, 1904–5, 16, p. 122.
ZWICK. *Centralbl. f. Bakt.*, I, Ref., 1910, 47, p. 219.